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THE HISTOLOGICAL, BIOCHEMICAL AND HEMATOLOGICAL ALTERATION IN ANABAS TESTUDINEUS (CUVIER) EXPOSED TO INSECTICIDE MONOCROTOPHOS.

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ABSTRACT

Monocrotophos is an organophosphorus insecticide widely used in agricultural fields for controlling varieties of insect pests. Extensive use of pesticides has led to contamination of water bodies thereby affecting the aquatic biota. This study was carried out to evaluate the possible histological, biochemical and hematological alterations in Anabas testudineus exposed to sublethal concentration of monocrotophos (45ppm). Histopathological changes in liver like distortion of hepatic parenchyma, pyknotic nuclei, leucocytic infiltration and in kidney like multifocal cloudy, cytoplasmic vacuolation, necrosis of hemopoietic tissues were observed. Biochemical analysis showed increased total tissue protein in the initial period of exposure and then depletion in later stage in hepatic and renal tissues accompanied with enhanced catalase activity. A decrease in total erythrocyte count, hemoglobin content and increased total leucocytes count was observed. The histological, biochemical and hematological alterations led to conclusion that monocrotophos has deleterious effects on Anabas testudineus and may jeopardize the health of other aquatic organisms.

KEY WORDS: Monocrotophos, Protein, Catalase, Erythrocyte, Leucocytes & Liver and Kidney

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Original Article

INTRODUCTION

In India, pesticides are used extensively in agricultural sector to control pest for improving crop production to meet the high food demand of fast growing population. These pesticides find their way into the aquatic environment mostly through runoff water from agricultural fields and cause various deleterious effects on aquatic biota. The organophosphate pesticides are extremely toxic to non-target species of freshwater fauna that damages the population dynamics, complex food web and food web energetic (Chandra et al., 2001). Bioaccumulations of pesticides affect the survival of fishes by disrupting the ecological relationships between organisms and loss of biodiversity (Abedi et al., 2013). Prolonged exposure of fishes to pesticides induces histopathological damages, biochemical changes and hematological alterations (Mishra et al., 2008; Faggio et al., 2014;Pandey et al., 2014; Ullah et al., 2014; Ullah and Zorriezahra, 2015; Gobi et al., 2018). Several researchers have shown pathological lesions in different tissues of fish due to various pesticide exposure (Cengiz and Unlu, 2006; Ogueji et al., 2013; Zahran et al., 2018).

Severe biochemical and enzymatic alterations have been observed due to secondary metabolites of pesticides in fishes (Rawat etal., 2002; Tiwary and Singh, 2009). Several researchers have reported reduction in tissue protein content under toxicity stress and this might be due to high protein hydrolytic activity because of increased protease activity (Muley et al., 2007; Prasanth and Neelagund (2008); Tiwary and Singh, 2009). The level and activity of antioxidant enzymes like catalase and glutathione peroxidase are affected by toxic pollutants and are used as biomarkers to assess the health of fish (Van der Oost et al., 2003). Hematological parameters are considered

potential biomarkers of pathological changes induced by toxicants in fishes (Talas and Gulhan, 2009). The effects of pesticides have been observed on blood parameters like decreased total erythrocyte count (TEC), hemoglobin (Hb) content and increased total leucocytes count (TLC) (Pereira et al., 2013; Shahi et al., 2013).

Hence, the present study was undertaken for making an integrated analysis of the histological, biochemical and hematological parameters of Anabas testudineus to evaluate the possible toxic effects of sub lethal concentration of monocrotophos for 10 and 20 days exposure.

MATERIALS AND METHODS

Anabas testudineus, commonly called climbing perch were collected from Central Institute of Fishery and Aquaculture (CIFA), Bhubaneswar, Odisha. The fishes were disinfected using 1% potassium permanganate solution. They were kept in large tanks (4'x2.5'x3') containing well aerated and dechlorinated water for 20 days to get acclimatized to laboratory conditions. The fishes were fed with fish pellets developed by CIFA for *Anabas*. The physico-chemical characteristics of water were carried out at regular intervals throughout the study period.

After acclimatization, forty five fishes of almost equal size and weight were segregated, divided into three groups i.e. each of fifteen fishes maintained in three separate tanks containing 150 liters of water each. In one of the tank (Tank 1), fishes were maintained in normal dechlorinated water served as control group and the other two tanks were experimental groups having sublethal concentration of monocrotophos (45 ppm). On every second day normal water in control group tank and pesticide mixed water in experimental group tanks were renewed. Fishes in one of the experimental tank (Tank 2) was exposed to monocrotophos for 10 days while the fishes of other experimental tank (Tank 3) exposed for 20 days.

To study the effect of monocrotophos, five fishes each of control group and experimental group were randomly selected and anaesthetized after 10 days of pesticide exposure. Blood was collected from the caudal peduncle of fishes of both control and experimental groups individually by using heparinized disposable syringes containing 0.5 mg ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The anticoagulant mixed blood was used for hematological studies. Total erythrocyte count (TEC), Hemoglobin content and total leucocytes count (TLC) were estimated by using an autoanalyzer. The same anaesthetized fishes were dissected under normal saline. The liver and kidneys were removed and fixed in aqueous Bouin's fluid for 24 hours. The tissues were dehydrated in a serial manner using alcohol, embedded in paraffin blocks and sectioned at 5 µm by using rotatory microtome. The tissue sections were spread on slide, stained using hematoxylin and eosin and mounted by using DPX. For biochemical studies, liver and kidney was removed and used for estimating total protein content following standard method of Bradford (1976) and catalase activity by Aebi (1983). All the above mentioned activities were repeated after 20 days of monocrotophos exposure.

Table 1: Physico-Chemical Characteristics of Water used for the Study

Parameters	Calculated Value
Temperature	30 ± 2^0
Turbidity	6.7 silica units
pH at 30^0C	7.4±0.05
Total hardness ($CaCO_3$)	198±6 (mg/L)
BOD	7 – 10 ppm
COD	Nil
Dissolved oxygen (DO)	5.5±0.5 mg/L

Statistical Analysis

Data obtained from replicates were used to calculate mean values. The hematological and biochemical data were subjected to one way analysis of variance (ANOVA) and the significance difference was set up at $P<0.001$ and $P<0.01$.

RESULTS AND ANALYSIS

Histoarchitectural Changes

Microscopic sections of kidney of control group fishes showed normal renal corpuscles having Bowmen's capsule with glomerular capillaries, proximal and distal tubules and hemopoietic cells figure 1 and 2). Fishes exposed to monocrotophos for 10 days showed histopathological changes. There was lesions in renal tissues with multifocal cloudy, cytoplasmic vacuolation, pyknotic nuclei, disintegrated cuboidal cells of glomeruli and cytological necrosis of hemopoietic tissues (figure 3 and 4). The result of the present study revealed severe histopathological alterations on exposure to pesticide for a prolonged period (20 days). There was inflammation of Bowman's capsule, severe vacuolization and necrosis of tubular epithelium characterized by karyorrhexis and karyolysis, acute necrosis of hemopoietic tissues and coalescence of adjacent renal tubules (figure 5 and 6). Interstitium was markedly infiltered with mononuclear cells in comparison to control group fishes (figure 6).

In the control group, the histoarchitecture of liver revealed the general histology of the organ. It exhibited systematic arrangement of parenchyma composed of hepatocytes arranged in diffused manner in branching tubules. Hepatocytes are polygonal with centrally located nuclei arranged as irregular cord like structures and separated by sinusoids (figure 7 and 8). Fishes exposed to monocrotophos for 10 days showed histological alterations like necrosis of hepatocytes, pyknotic nuclei, dilation of sinusoids, leucocytic infiltration and large vacuoles within the hepatocytic cytoplasm figure 9 and 10). Prolonged exposure (20 days) to monocrotophos induced severe histopathological changes like dystrophic lesions in hepatocytes, nuclear fragmentation, wider intercellular spaces due to connective tissue damage and hemorrhages at several points (figure 11 and 12). Many of the nuclei became pyknotic with gradual process of cytolysis and large vacuoles appeared within cytoplasm resulting from cell membrane degeneration (figure 11).

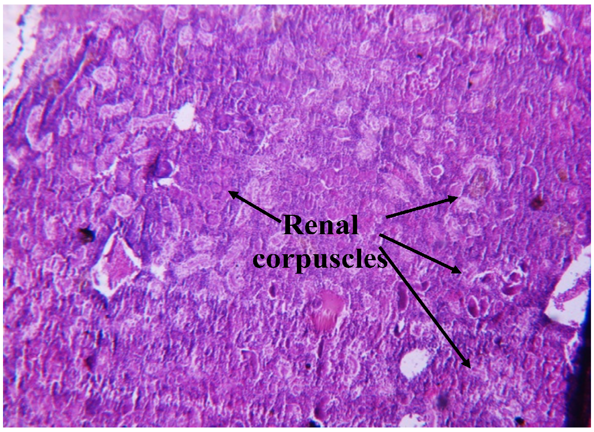

Figure 1: Section of Kidney of Control *Anabas* showing Normal Histoarchitecture, 100X

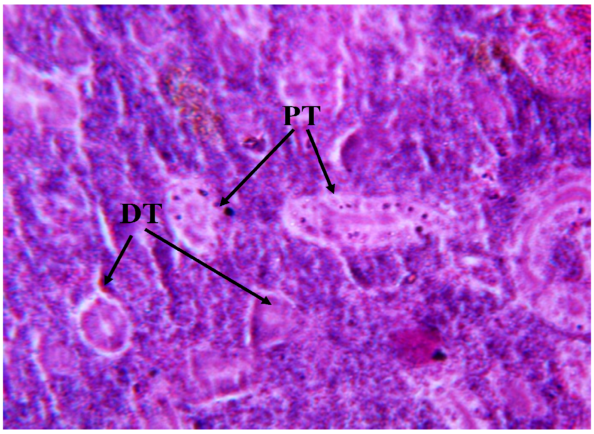

Figure 2: Section of Kidney of Control *Anabas* showing Normal Proximal Tubules (PT) and Distal Tubules (DT), 400X

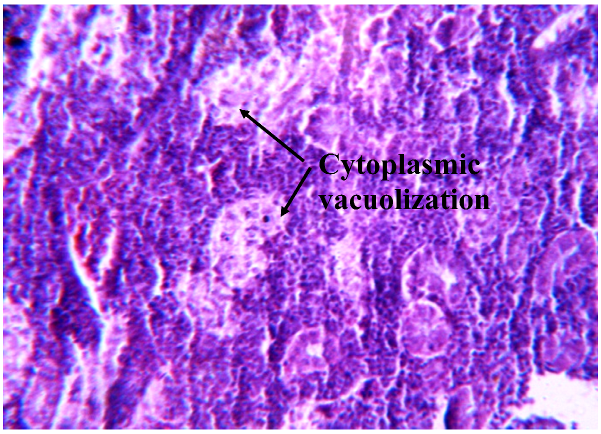

Figure 3: Section of Kidney of 10th Day Treated *Anabas* Showing Cytplasmic Vacuolization, 400X

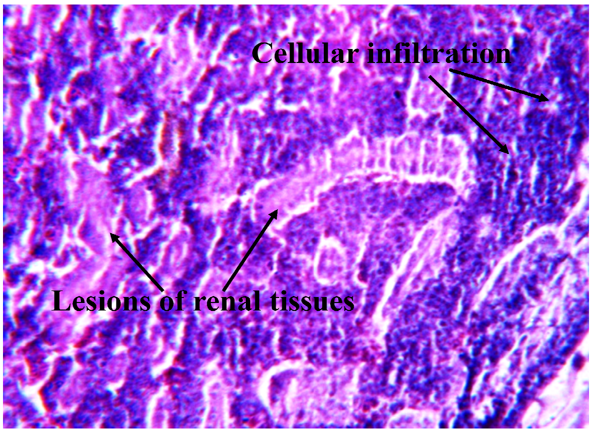

Figure 4: Section of Kidney of 10th Day treated *Anabas* showing Cellular Infiltration and Lesions of Renal Tissues, 400X

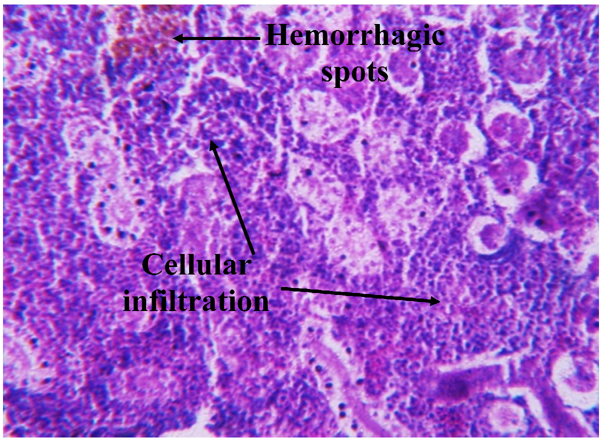

Figure 5: Section of Kidney of 20th Day Treated *Anabas* showing Cellular Infiltration and Hemorrhagic Spots, 400X

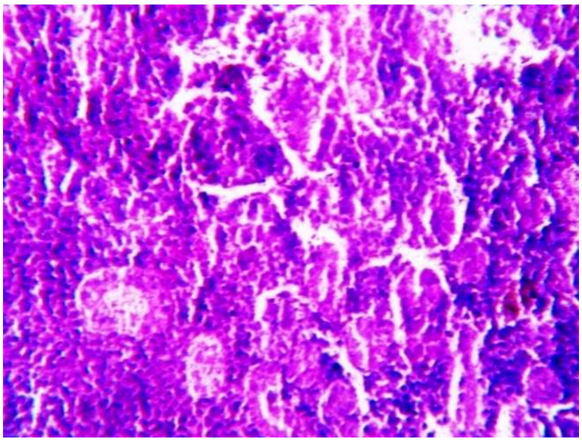

Figure 6: Section of Kidney of 20th Day Treated *Anabas* showing Cytoplasmic Vacuolization, Pycnotic nuclei and Disintegration of Renal Tissues, 400 X

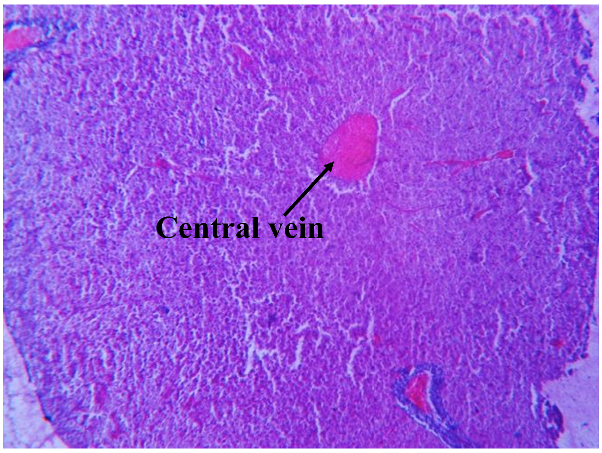

Figure 7: Section of Liver of Control *Anabas* showing Vein and Hepatocytes 100X

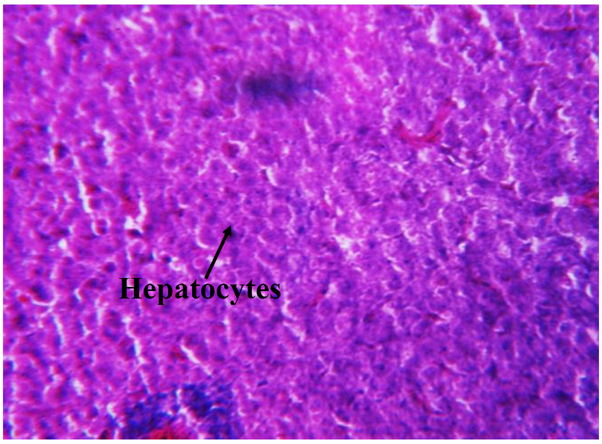

Figure 8: Section of Liver of Control *Anabas* showing Hepatocytes 400X

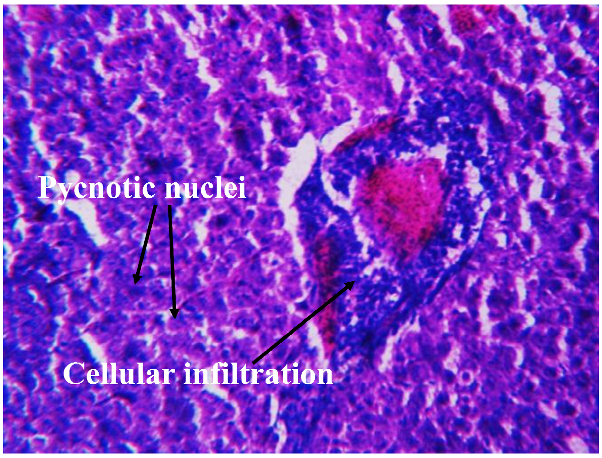

Figure 9: Section of Liver of 10th Day Treated *Anabas* showing Pycnotic Nuclie and Cellular Infiltration 400X

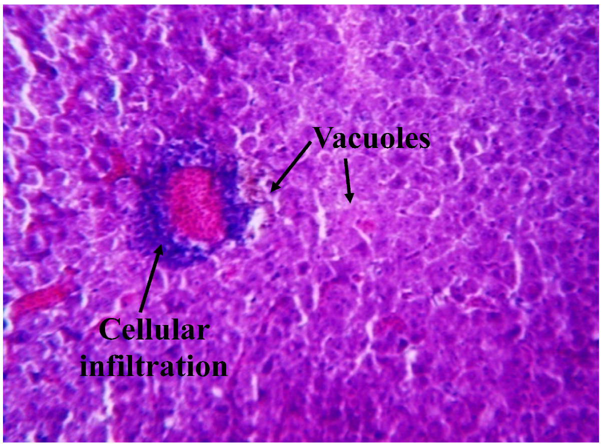

Figure 10: Section of Liver of 10th Day Treated *Anabas* showing Vauolization, and Cellular Infiltration 400X

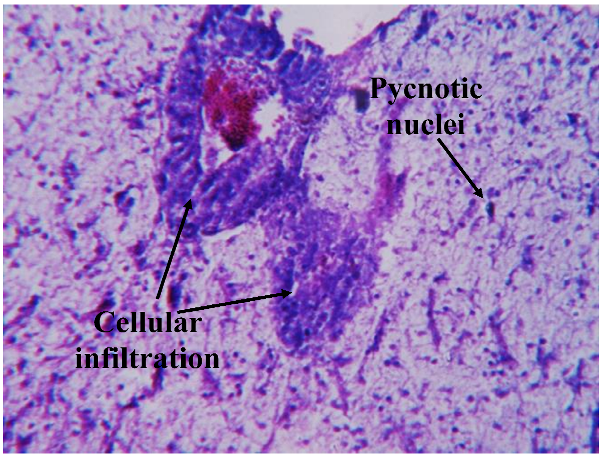

Figure 11: Section of Liver of 20th Day Treated *Anabas* showing Heavy Disintegration of Hepatocytes 400X

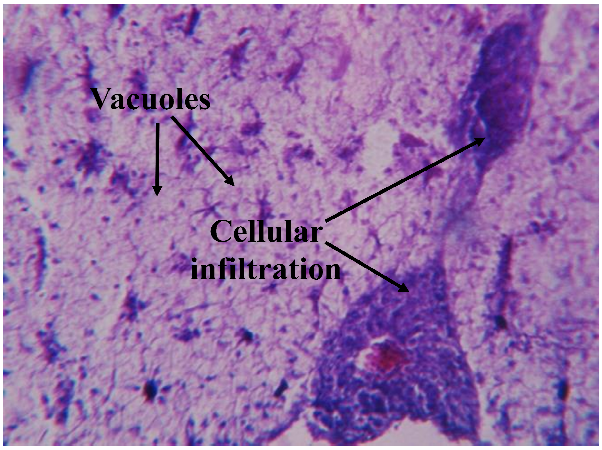

Figure 12: Section of Liver of 20th Day Treated *Anabas* showing Cytoplasmic Vacuolization, Cellular Infiltration and Pycnotic Nuclei 400X

Biochemical Changes

Table 2: Effect of Monocrotophos on Protein Content of Anabas Testudineus

Treatment	Tissues	Group	N	Mean	SEM	SD	MD	df	t	Significance
10 days	Kidney	control	5	167.6000	2.37576	5.31235	-123.80000	4	-27.008	.000
		experimental	5	291.4000	5.00943	11.20143				
	Liver	control	5	145.8900	2.07944	4.64977	-47.91000	4	-12.212	.000
		experimental	5	193.8000	1.87977	4.20329				
20 days	Kidney	control	5	171.4000	1.66506	3.72319	94.60000	4	58.426	.000
		experimental	5	76.8000	1.76095	3.93761				
	Liver	Control	5	152.9000	2.32451	5.19777	56.67000	4	32.891	.000
		experimental	5	96.2300	1.29951	2.90580				

Table 3: Effect of Monocrotophos on Catalase Activity of Anabas Testudineus

Treatment	Tissues	Group	N	Mean	SEM	SD	MD	df	t	Significance
10 days	Kidney	control	5	161.0200	2.38726	5.33808	-22.50000	4	-5.572	.005
		experimental	5	183.5200	1.94829	4.35652				

	Liver	control	5	901.8700	4.21224	9.41885	-672.08000	4	-94.861	.000
		experimental	5	1573.9500	4.21224	9.41885	-672.08000			
20 days	Kidney	control	5	170.7000	2.75645	6.16362	-88.44000	4	-42.258	.000
		experimental	5	259.1400	2.99887	6.70567				
	Liver	control	5	914.3700	18.11338	40.50274	-1503.2300	4	-16.445	.000
		experimental	5	2417.6000	75.96772	169.8690				

The results of the present study showed an initial increase in total tissue protein in renal and hepatic tissues of Anabas testudineus in experimental group fishes after 10 days of exposure to monocrotophos in comparison to control group fishes (table 2; figure 13). The mean total protein content of renal tissues of experimental fishes after 10 days of exposure was 291.4 mg/g which was significantly (P<0.001) higher than control fishes (167.6 mg/g) with an increase of 73.8%. Similar trend was observed for hepatic tissues. The total protein content in pesticide exposure fishes (193.8 mg/g) was significantly (P<0.001) higher than control group fishes (145.89 mg/g) with an increase of 32.8%. However, after prolonged exposure of fishes to monocrotophos for 20 days, a considerable reduction in protein content was recorded both in renal and hepatic tissues (table 2; figure 13). After 20 days of exposure, total protein content in renal tissues of control group fishes (171.4 mg/g) was significantly (P<0.001) higher than experimental group fishes (76.8 mg/g) with a decrease of 55%. Similarly in case of hepatic tissue, the total protein content of control group fishes (152.9 mg/g) was significantly (P<0.001) higher than pesticide exposed fishes (96.23 mg/g) with a decrease of 37%.

Catalase is an antioxidant enzyme catalyzes the decomposition of H_2O_2 to molecular O_2 and H_2O. The data of the present study showed a time dependent increase in catalase activity in renal and hepatic tissues of *Anabas testudineus* exposed to monocrotophos with respect to control (table 3; figure 14). After 10 days of exposure, the catalase activity in renal tissues of experimental group fishes (183.52 µmol/mg protein) was significantly (P<0.01) higher than control group fishes (161.02 µmol/mg protein). Similarly, hepatic tissues of monocrotophos fishes (1573.95 µmol/mg protein) was significantly (P<0.01) higher than control (901.87 µmol/mg protein). A further increase in catalase activity in renal and hepatic tissues was observed after 20 days of exposure to monocrotophos. In case of prolonged exposure, the catalase activity in renal tissues (259.14 µmol/mg protein) was significantly (P<0.001) higher than control (170.7 µmol/mg protein). The hepatic tissues of pesticide exposed fishes (2417.6 µmol/mg protein) was significantly (P<0.001) higher than control (914.37 µmol/mg protein).

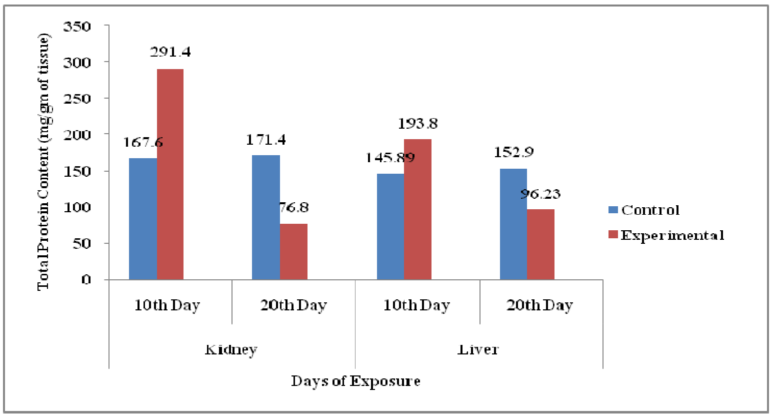

Figure 13: Effect of Monocrotophos on Protein Content of Anabas Testudineus.

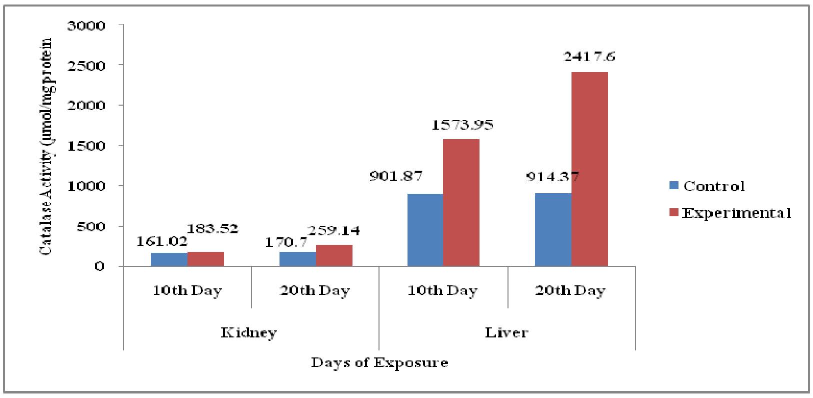

Figure 14: Effect of Monocrotophos on Catalase Activity of Anabas Testudineus.

Hematological Changes

Hematological parameters are often used to predict the physiological state of fish. Significant changes were induced by the monocrotophos toxicity in different hematological parameters of Anabas testudineus (table 4, 5 and 6; figure 15, 16 and 17). TEC ($x10^6/mm^3$) in fishes exposed to pesticide for 10 days was lower than control. A significant (P<0.01) decrease in TEC was observed when fishes were exposed to pesticide for 20 days than control (table 4; figure 15). Similarly hemoglobin content (g%/dL) was significantly (P<0.01) reduced in pesticide exposed fishes for 10 and 20 days than control (table 5; figure 16). Whereas total lecocytes count ($x10^3/mm^3$) showed a significant (P<0.01) increasing trend in pesticide exposed fishes for 10 and 20 days in relation to control indicating hypersensitivity of leucocytes to monocrotophos (table 6; figure 17).

Table 4: Effect of Monocrotophos on TEC ($X10^6/Mm^3$) of Anabas Testudineus

Treatment	Group	N	Mean	SEM	Sd	Md	df	t	Significance
10 days	control	5	2.7800	.18385	.18385	.59000	4	2.722	.053
	experimental	5	2.1900	.08349	.08349				
20 days	control	5	2.8500	.17114	.17114	1.1200	4	6552	.003
	experimental	5	1.7300	.06738	.06738				

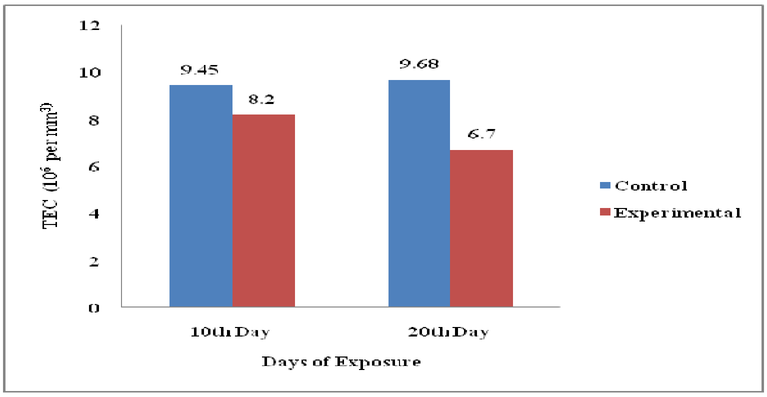

Figure 15: Effect of Monocrotophos on TEC ($X10^6/Mm^3$) of Anabas Testudineus.

Table 5: Effect of Monocrotophos on Hemoglobin (Gm%/Dl) of Anabas Testudineus

Treatment	Group	N	Mean	SEM	SD	MD	df	t	Significance
10 days	control	5	9.4500	.16985	.37980	1.25000	4	5.978	.004
	experimental	5	8.2000	.18836	.42119				
20 days	control	5	9.6800	.19905	.44508	2.98000	4	7.337	.002
	experimental	5	6.7000	.24793	.55439				

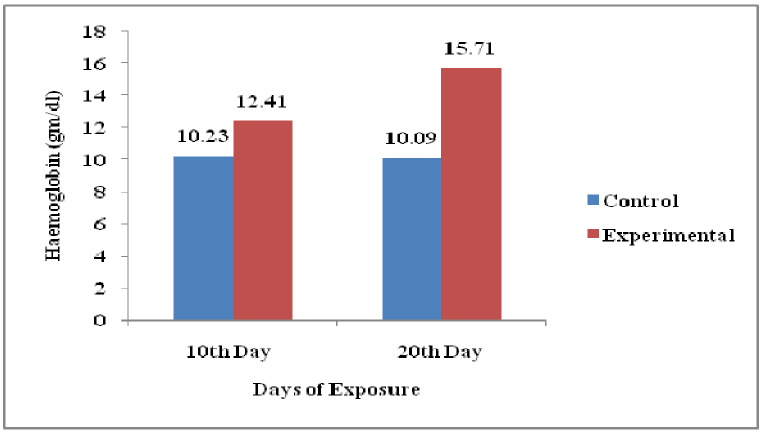

Figure 16: Effect of Monocrotophos on Hemoglobin (Gm%/Dl) of Anabas Testudineus.

Table 6: Effect of Monocrotophos on TLC ($X10^3/Mm^3$) of Anabas Testudineus

Treatment	Group	N	Mean	SEM	SD	MD	df	t	Significance
10 days	control	5	10.2300	.12958	.28974	-2.18000	4	-5.976	.004
	experimental	5	12.4100	.25618	.57284				
20 days	control	5	10.0900	.09965	.22282	-5.62000	4	-11.218	.000
	experimental	5	15.7100	.41135	.91981				

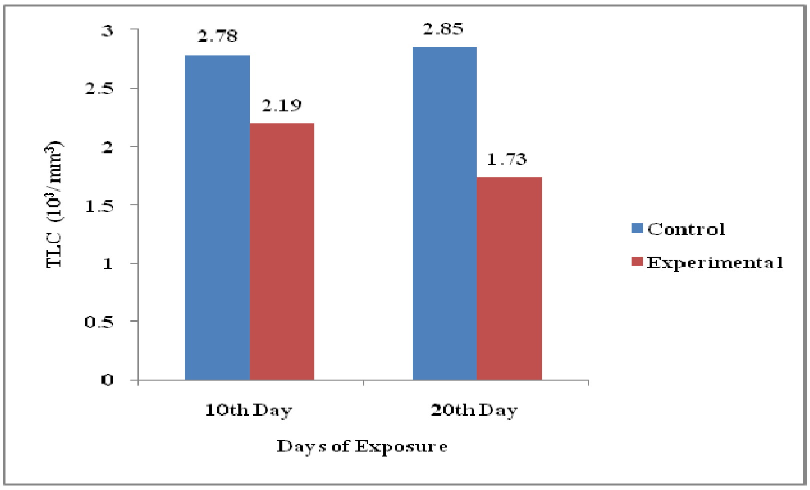

Figure 17: Effect of Monocrotophos on TLC ($X10^3/Mm^3$) of Anabas Testudineus

DISCUSSIONS

Histopathological changes have been widely used as Biomarkers for monitoring the effects of pollutants on specific target organs like liver, kidney and gills that are responsible for vital functions (Gernhoferet al., 2001; Joseph and Raj, 2011 and Deb and Das 2013). The alterations found in these organs on exposure to chemicals like pesticides are normally easier to identify than functional ones and serve as markers of damage to animal health (Ceingiz and Unlu, 2003; Fanta et al., 2003; Bartoskova et al., 2013 and Gobi et al.,2018).

Histopathological alterations in the renal tissues of Anabas testudineus in the present study showed renal toxicity of monocrotophos. The results of the present investigation indicated that exposure to monocrotophos induced lesions in renal tissues with multifocal cloudy, hydropic degenerations with acute cytological necrosis, cytoplasmic vacuolation, and

pycnotic nuclei. Similar pathological changes have earlier been reported by Dumitrescu et al. (2010) in Danioreria exposed to octylphenol, and Olufayo and Alade (2012) in Chanapunctatus. Satyanarayan et al. (2012) in Cyprinuscarpio reported that on exposure to chlorinated hydrocarbon pesticides (Dieldrin, BHC and DOT) there was complete disintegration of cuboidal cells of epithelial layer of glomeruli, acute cytological necrosis of hemopoietic tissue and vacuolation. Similar effects have been observed in the kidney of Anabas testudineus on exposure to monocrotophos in the present study. The results of present investigation showed shrinkage of glomeruli which may be due to constriction of capillaries because of monocrotophos concentration in the blood leading to decrease in glomerular filtration. Similar findings have also been reported by Satyanarayanet al., (2012) in Cyprinuscarpio. Prolonged exposure to monocrotophos (20 days) in the present study resulted in pronounced inflammation of Bowman's capsule, severe vacuolization of tubular epithelium followed by pycnosis, necrosis in hemopoietic tissues and coalescence of adjacent renal tubules and infiltration of interstitium with mononuclear cells. These recorded results of the present study were similar to the observations reported by Tilak et al. (2005) in Catlacatla and Cirrhinusmrigala and Kumar et al. (2018) in Anabas testudineus.

The liver in fish like other animals is the organ of detoxification suffers acute morphological alternations on exposure to pesticides. Most important function of the liver is the secretion of bile. It acts as an excretory organ and eliminates waste products, toxic substances and metallic poisons. In normal histoarchitecture, the parenchyma of the liver is composed of hepatocytes which exhibit a diffused or radial organization in branching tubules. Several researchers have investigated the histological alterations in liver of fish on exposure to different chemicals. Mishra et al., (2006) demonstrated histoarchitectural alternations in liver of Channapunctatus on sublethal exposure to car baryl like hypertrophy with vacuolation of hepatocytes, pyknotic nuclei, dilation of blood sinusoids and focal necrosis. Joshi et al. (2007) in Heteropneustesfossilis and Dumitrescu et al. (2010) in Daniorerio observed extensive degeneration of cytoplasm and shrinkage of the hepatocytes with pyknosis of nuclei and breakdown of blood sinusoids when subjected to hexachlorocyclohexane, dipterex and octylphenol toxicity respectively. In the present study marked histopathological lesions were recorded in the liver of Anabas testudineus exposed to sublethal concentration of organophosphate pesticide monocrotophos. There was pronounced swelling of hepatocytes, vacuolated cytoplasm, pyknotic nuclei, dilation of blood sinusoids and leucocytic infiltration**.** These results are in agreement with the data published by Sarkaret al. (2001) in Clariasgariepinus exposed to organophosphorous insecticide (Hastathion), Tilaket al. (2005) in Catlacatla and Kunjammaet al. (2008) in Oreochromismossambicus (Tilapia) exposed to chlorpyrifos, Gawishet al., (2011) in Nile tilapia on exposure to lorsban, and in Channapunctatus by Paithane et al. (2012) on exposure to dimethoate and Devi and Mishra (2013) on exposure to cypomethrin. Their results showed that pesticides produced histopathological changes in the liver represented by pyknotic nucleus, protein precipitation, hepatic tissue necrosis, cytoplasmic vacuolation of the hepatocytes, damaged blood sinusoids and inflammatory leucocyticinfiltration. The results of the present investigation indicated severe histopathological changes with increased duration of time i.e., 20 days exposure in comparison to 10 days exposure like hemorrhage, degeneration and dystrophic lesions in hepatocytes, nuclear fragmentation, vacuolization and wider intercellular spaces due to connective tissue damage. This severity may be due to fact that, as liver is the major site of detoxification and it is expected that the toxicant pesticide would reach there in abundance for detoxification and removal. Similar duration dependant histological alterations have been reported by Karmakar et al. (2015) in Labeorohita, Begum and Mishra (2015) in Heteropneustesfossilis, Maksymiv et al. (2015) in Carrasiusauratus exposed to different pesticides. The appearance of pyknotic nuclei as observed in the present study indicated that the cells became hypofunctional

resulting in focal necrosis. As liver cells are involved in the metabolic transformation of the pesticide, monocrotophos causing architectural and functional changes probably led to focal necrosis. This finding of the present study is almost similar to those by Rodrigues and Fanta (1998) in Heteropneustesfossilis and Brachydaniorerio exposed to organophosphate insecticide malathion and dimethoate.

Proteins an important organic substance for tissue building and also plays an important role in energy production during stress period in animals. An attempt has been made in the present study to evaluate the toxic effect of monocrotophos on protein metabolism in terms of total tissue protein in Anabastestudineus. Several investigators revealed that exposure to sublethal doses of different pesticides significantly caused hypoproteinemiain most of the fish tissues (Tilak and Yacob (2002); Vishal(2004); Kalender et al. (2005); Venkataramana et al. (2006); Rohankaret al. (2012) and Nagaraju and Rathnamman (2013). Depletion in total tissue protein may be due to increased proteolytic activity and possible utilization of their products for metabolic purposes under toxicant stress (Jenkins et al., 2003; Muley et al., 2007; Remia et al., 2008; Tiwary and Singh, 2009; Bibi et al., 2014). Tripathi and Yadav (2015) suggested that reduced total tissue protein might be due to low protein synthesis under toxic stress condition. Some researchers like Tripathiet al. (2006) and Gawish et al. (2011) have recorded increase in total protein content in the liver of Clariasbatrachus exposed to cypermethrin and in Nile tilapia exposed to lorsban respectively. The present study revealed a significant increase in the total protein content in the liver and kidney of Anabas testudineus exposed to monocrotophos during initial stage of experimental period (10 days) and then hypoproteinemia was observed upon prolonged exposure (20 days**).** The result of the present study is in conformity with the observations recorded by Khareet al. (2000) in Clariasbatrachus exposed to malathion and Bose et al. (2011) in Oreochromisniloticus exposed to thiamethoxam. The initial hyperproteinemia in liver and renal tissues may be for counteracting the effect of toxicant and required for repair of damage cell organelle and tissue regeneration. The rapid decline in total protein observed during prolong exposure may be result of increased proteolysis to meet the energy demand in stressful condition and synthesis of lipoproteins for repairing cell damages.

Activities of enzymes are important biochemical indicators of health in fish. The enzyme catalase an antioxidant enzyme serves to protect cells from the toxic effect of H_2O_2 by catalyzing it to H_2O and O_2. Sayeedet al. (2003) reported reduced catalase activity in liver, kidney and gill of Chanapunctatus exposed to deltamethrin. Tripathi and Singh (2013) observed alphamethrin induced reduction in catalase activity in Chanapunctatus. They suggested that depletion in catalase activity may be due to binding of toxicant to the enzyme molecule or inhibiting the enzyme synthesis. However, the findings of present study are not in agreement with Sayeed et al. (2003), Tripathi and Verma (2004), Sulfath et al. (2013) and Tripathi and Singh (2013). The results of the present study showed a significant increase in catalase activity in both liver and kidney of Anabas testudine us after exposure to monocrotophos. Prolong exposure enhanced catalase activity in hepatic and renal tissues of treated fishes in comparison to fishes of control group**.** This increase in catalase activity may be due to stimulation of antioxidant defense system in liver and renal tissues to combat the intense production of free radicals indicating an effective protection against H_2O_2 a powerful oxidizing agent. Similar findings on antioxidant enzymes have been reported by Atifet al. (2005) in Channapunctatus exposed to deltamethrin, Moraes et al. (2007) in Leporinusotusidens exposed to clomazone; Prasanth and Neelagund (2008) in Cirrhinusmrigala exposed to cypermethrin and Pereira et al. (2013) in Prochilodusline at us. The augmented activity of catalase also reflects the peroxisome proliferation due to activation of antioxidant pathways (Syakalima et al., 2006).

In recent years hematological variables have been recognized as valuable indicators for monitoring the toxicological effects of pollutants and environmental stress in fish (Talas and Gulhan, 2009). Researchers like Ayoola (2008) in Oreochromisniloticus exposed to herbicide glyphosate, Ada et al. (2012) in Oreochromisniloticus exposed to parquet herbicide and Sepperumul and Saminathan (2013) in Oriochromismossambicus treated with diethylphthalate observed increase in mean RBCs count and hemoglobin content. Ayoola (2008), and Ada et al. (2012) in their study have shown that there was a significant decrease in erythrocyte count with increasing dose of pesticide to a point and then increased with further increase in concentration. The results of the present study disagree with the observations of above investigators. The findings of the present investigation showed that the monocrotophos exposure inflicted a drastic reduction in the total erythrocyte count (TEC) and hemoglobin content as compared to fishes of control group ($P<0.01$). The reduction was duration dependent. The increase in exposure duration (20days) was associated with a decrease in total erythrocyte count and hemoglobin concentration**.** The present findings is in consistent with that of Jenkins et al. (2003); Johal and Grewal (2004); Shah and Altindag (2004); Murugan (2006); Jaffar Ali and Jaya Rani (2009); Talas and Gulham (2009); Kumar et al. (2011) and Shahi et al. (2013). Decrease in hemoglobin concentration as observed in the present study may be due to the decrease rate of production of erythrocytes or an increased loss of these cells. Shahi and Singh (2014) suggested that significant decrease in Hb concentration may be due to either an increase in the rate at which Hb is destroyed or to a decrease in the rate of Hb synthesis. The possible reason for impaired erythropoiesis may be due to direct effect of monocrotophos on the hemopoietic center i.e. kidney as observed in the present study. However, Sarochet al. (2012) attributed decreased RBCs count to accelerated erythroclasia due to altered membrane permeability and defective iron metabolism or impaired intestinal uptake of iron due to mucosal lesions.

CONCLUSIONS

Anabas testudineus exposed to monocrotophos exhibited significant increase in total leucocytes count (TLC). With increase of exposure time (20 days), there was further rise in TLC in comparison to control fishes. This finding is corroborated by the results reported by Joshi and Deep (2002) in Clariasbatrachus exposed to Lindane and Malathion, Chindah et al. (2004) in Tilapia guinensis exposed to chlorpyrifos, Sekhar et al. (2011) in Mystusvittatus exposed to monocrotophos, Samajdar and Mandal (2015) in Labeorohitaexposed to chlorpyrifos. However, David et al. (2015) observed an initial increase in TLC and decrease in later stage in Cirrhinusmrigala on exposure to deltamethrin. They attributed initial increase might be due to direct stimulation of immunological defense due to tissue destruction by deltamethrin and decreased TLC in later stage might be due to series of changes in immunological set up of the fish under pesticide stress. The increase in total leucocytes count observed in the present investigation could be attributed to a stimulation of the immune system to counter the deleterious effect of monocrotophos.

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